Molecular Genetics and Metabolism Reports 11 (2017) 92–93

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Correspondence

# Next-generation sequencing corroborates a probable *de novo GNPTG* variation previously detected by Sanger sequencing

*Keywords:*
Mucolipidosis III gamma
Next-generation sequencing
*De novo* alteration

Letter to the Editor

Mucolipidosis III (MLIII) is an autosomal recessive disease caused by pathogenic variations in the *GNPTAB* (MLIII alpha/beta) or *GNPTG* (MLIII gamma) genes. *GNPTAB* and *GNPTG* encode, respectively, the α/β and γ subunits of GlcNAc-1-phosphotransferase, the enzyme responsible for catalyzing the addition of a mannose-6-phosphate residue to lysosomal hydrolases, allowing their entry into lysosomes [1,2].

In 2014, our group published a paper describing an MLIII gamma patient, born to a non-consanguineous couple, who carried the variations c.244_247dupGAGT at exon 3 and 328G > T at exon 5, both detected by Sanger sequencing of *GNPTG*. Analysis of parental DNA using the same technique showed c.328G > T in the father and no alterations in the mother, despite using different pairs of primers and different tissues. Maternity was confirmed, and c.244_247dupGAGT was thus considered a *de novo* mutation [3].

Next-generation sequencing (NGS) has proven more sensitive than Sanger sequencing for the detection of mosaicism when the variants are located in exons, as in the present case [4–10]. However, NGS has several limitations, such as incomplete acceptable coverage of coding regions due to regions rich in CpG islands or repetitive sequences [11]. Thus, we decided to analyze DNA samples obtained from the blood of the patient and her mother using the Ion 314™ Chip Kit v2 and sequence *GNPTG* with the Ion PGM Hi-Q Sequencing Kit (Thermo Fisher Scientific). The fragment of *GNPTG* where c.[244_247dupGAGT] is located was amplified 406 and 1047 times in the maternal and patient samples, respectively. No alteration was identified in the maternal sample, while both were identified in the patient, confirming our previous findings. As we failed to detect mosaicism using this technique, the possibility of a *de novo* event is even higher. Our findings corroborate the hypothesis that *de novo* variations may be more frequent than expected [12], and speak strongly in favor of always investigating the parents of a child presenting with an autosomal recessive disease in order to confirm heterozygous status.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgments

This study was funded by the Brazilian National Council for Scientific and Technological Development (CNPq - 484259/2012-1 and 308816/2013-7), the Brazilian Federal Agency for the Support and Evaluation of Graduate Education (CAPES - 484259/2012-1), and the Hospital de Clínicas de Porto Alegre Research Incentive Fund (FIPE-HCPA - 12-0276).

## References

[1] S.S. Cathey, M. Kudo, S. Tiede, A. Raas-Rothschild, T. Braulke, M. Beck, H.A. Taylor, W.M. Canfield, J.G. Leroy, E.F. Neufeld, V.A. McKusick, Molecular order in mucolipidosis II and III nomenclature, Am. J. Med. Genet. A 143A (2008) 2106–2112, http://dx.doi.org/10.1002/ajmg.a.

[2] T. Braulke, A. Raas-Rothschild, S. Kornfeld, I - Cell Disease and Pseudo - Hurler Polydystrophy: Disorders of Lysosomal Enzyme Phosphorylation and Localization, Online Metab. Mol. Bases Inherit. Dis., 2015 1–31, http://dx.doi.org/10.1036/ommbid.167.

[3] R.V. Velho, T. Alegra, F. Sperb, N.F. Ludwig, M.L. Saraiva-Pereira, U. Matte, I.V.D. Schwartz, A de novo or germline mutation in a family with mucolipidosis III gamma: implications for molecular diagnosis and genetic counseling, Mol. Genet. Metab. Reports. 1 (2014) 98–102, http://dx.doi.org/10.1016/j.ymgmr.2014.01.002.

[4] L. Liu, Y. Li, S. Li, N. Hu, Y. He, R. Pong, D. Lin, L. Lu, M. Law, Comparison of next-generation sequencing systems, J. Biomed. Biotechnol. 2012 (2012) http://dx.doi.org/10.1155/2012/251364.

[5] A. Rohlin, J. Wernersson, Y. Engwall, L. Wiklund, J. Björk, M. Nordling, Parallel sequencing used in detection of mosaic mutations: comparison with four diagnostic DNA screening techniques, Hum. Mutat. 30 (2009) 1012–1020, http://dx.doi.org/10.1002/humu.20980.

[6] H. Tuhan, A. Anik, G. Catli, H. Onay, A. Aykut, A. Abaci, E. Bober, A novel mutation in steroidogenic factor (SF1/NR5A1) gene in a patient with 46 XY DSD without adrenal insufficiency, First Int. J. Androl. 1 (2016) 1–5, http://dx.doi.org/10.1111/and.12589.

[7] J. Hague, I. Delon, K. Brugger, H. Martin, S. Abbs, S.-M. Park, Molecularly proven mosaicism in phenotypically normal parent of a girl with Freeman-Sheldon Syndrome caused by a pathogenic *MYH3* mutation, Am. J. Med. Genet. A 170 (2016) 1608–1612, http://dx.doi.org/10.1002/ajmg.a.37631.

[8] D.Z. Bar, M.F. Arlt, J.F. Brazier, W.E. Norris, S.E. Campbell, P. Chines, D. Larrieu, S.P. Jackson, F.S. Collins, T.W. Glover, L.B. Gordon, A novel somatic mutation achieves partial rescue in a child with Hutchinson-Gilford progeria syndrome, J. Med. Genet. (2016) http://dx.doi.org/10.1136/jmedgenet-2016-104295 jmedgenet-2016-104295.

[9] A. Baurand, S. Falcon-Eicher, G. Laurent, E. Villain, C. Bonnet, C. Thauvin-Robinet, C. Jacquot, J.-C. Eicher, J.-B. Gourraud, S. Schmitt, S. Bézieau, M. Giraud, S. Dumont, P. Kuentz, V. Probst, A. Burguet, F. Kyndt, L. Faivre, Incomplete Timothy syndrome secondary to a mosaic mutation of the *CACNA1C* gene diagnosed using next-generation sequencing, Am. J. Med. Genet. A 1–6 (2016) http://dx.doi.org/10.1002/ajmg.a.38045.

[10] L. Qin, J. Wang, X. Tian, H. Yu, C. Truong, J.J. Mitchell, K.J. Wierenga, W.J. Craigen, V.W. Zhang, L.J.C. Wong, Detection and quantification of mosaic mutations in disease genes by next-generation sequencing, J. Mol. Diagn. 18 (2016) 446–453, http://dx.doi.org/10.1016/j.jmoldx.2016.01.002.

[11] A. Fernández-Marmiesse, M. Morey, M. Pineda, J. Eiris, M.L. Couce, M. Castro-Gago, J.M. Fraga, L. Lacerda, S. Gouveia, M.S. Pérez-Poyato, J. Armstrong, D. Castiñeiras, J.A. Cocho, Assessment of a targeted resequencing assay as a support tool in the diagnosis of lysosomal storage disorders, Orphanet J. Rare Dis. 9 (2014) 59, http://dx.doi.org/10.1186/1750-1172-9-59.

[12] J.M. Goldmann, W.S.W. Wong, M. Pinelli, T. Farrah, D. Bodian, A.B. Stittrich, G. Glusman, L.E.L.M. Vissers, A. Hoischen, J.C. Roach, J.G. Vockley, J.A. Veltman, B.D. Solomon, C. Gilissen, J.E. Niederhuber, Parent-of-origin-specific signatures of de novo mutations, Nat. Genet. 48 (2016) http://dx.doi.org/10.1038/ng.3597.

http://dx.doi.org/10.1016/j.ymgmr.2017.02.002

Nataniel Floriano Ludwig
Fernanda Sperb-Ludwig
*Graduate Program in Genetics and Molecular Biology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
*BRAIN Laboratory, Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil*

Renata Voltolini Velho
*Section of Biochemistry, Children's Hospital, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*

Ida Vanessa D. Schwartz
*Graduate Program in Genetics and Molecular Biology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
*BRAIN Laboratory, Hospital de Clinicas de Porto Alegre, Porto Alegre, Brazil*
*Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil*
Corresponding author at: Rua Ramiro Barcelos, 2350, Porto Alegre, Brazil.
*E-mail address:* ischwartz@hcpa.edu.br.

16 February 2017